Published in final edited form as:
Nat Rev Microbiol. 2010 September ; 8(9): 634–644. doi:10.1038/nrmicro2405.

A field guide to bacterial swarming motility

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Abstract

How bacteria regulate, assemble, and rotate flagella to swim in liquid media is reasonably well understood. Much less is known, however, about how some bacteria also use flagella to move over the tops of solid surfaces in a form of movement called swarming. As the focus of bacteriology changes from planktonic to surface environments, interest in swarming motility is on the rise. Here I review the requirements that define swarming motility in diverse bacterial model systems including an increase in the number of flagella per cell, secretion of a surfactant to reduce surface tension for spreading, and movement in multicellular groups rather than as individuals.

Bacteria have traditionally been viewed as unicellular organisms that grow as dispersed individuals in a planktonic environment. Recently, this view has begun to change with increasing awareness of the role of biofilms in which sessile bacteria secrete an extracellular matrix and aggregate as multicellular groups. Surface-associated bacteria have another option besides sessile aggregation; sometimes the bacteria become highly motile and migrate over the substrate, a process known as swarming. Biofilm research has renewed interest in bacterial swarming motility that is often oppositely regulated and antagonistic to biofilm formation[1].

Swarming motility is operationally defined as a rapid multicellular bacterial surface movement powered by rotating flagella[2] (Figure 1). Although simple, accurate, and mechanistically meaningful, the definition does not do justice to the wide array of phenotypes associated with swarming motility, nor does it emphasize all that remains unknown about this behavior. Furthermore, despite the simplicity of the definition, it is important to acknowledge the common field-specific misnomers (Box 1) and distinguish swarming from behaviors such as swimming, twitching, gliding, and sliding that can occur within, or on top of, solid surfaces[3] (Figure 1).

Swimming motility is a mode of bacterial movement powered by rotating flagella but, unlike swarming motility, takes place as individual cells moving in liquid environments. Twitching motility is surface motility powered by the extension and retraction of type IV pili that confers slow cell movement often with a jerky or “twitchy” appearance[4]. Gliding motility is a catch-all definition for active surface movement that occurs along the long axis of the cell without the aid of either flagella or pili. Gliding seems to have evolved independently in multiple lineages but generally involves the cell body moving through focal adhesion complexes that bind to the substrate[5]. Sliding motility is a passive form of surface spreading that does not require an active motor[2], but instead relies on surfactants to reduce surface tension enabling the colony to spread away from the origin driven by the outward pressure of cell growth. Furthermore, sliding is easily mistaken for swarming

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motility and can occur when the flagella are disrupted in bacteria that would normally swarm[6,7,8,9,10].

This review will introduce the phenomenon of swarming motility from a practical standpoint, synthesize the cellular requirements and phenotypes associated with swarming from diverse model organisms, and discuss some of the mysteries and controversies associated with this type of bacterial cell motility.

Studying swarming motility in the laboratory

Swarming motility seems to be narrowly conserved in the Bacterial domain and is currently restricted to three families (Figure 2). The reported number of swarming species is almost certainly an underestimate because swarming motility is often inhibited by standard laboratory media and genetically abolished during the domestication of commonly-used laboratory strains[11,12,13,14]. Selection against swarming may be due to evolutionary forces when surface motility provides no advantage in unstructured laboratory environments[15]. Alternatively, bacteria that spread promiscuously over plates are rarely welcomed by geneticists and selection against swarming may be artificial in favor of small, compact colonies.

Swarming motility generally requires an energy-rich, solid medium but the specific conditions that support swarming depend on the organism being considered. Some bacteria like *Bacillus subtilis* swarm on a wide range of energy-rich media whereas other bacteria like *Salmonella enterica* and *Yersinia entercolitica* require the presence of particular supplements like glucose[16,17,18]. Swarming is promoted by high growth rates which may account for the requirement for energy-rich conditions[12,19,20]. Although some bacteria can swarm over nearly any agar surface, most swarming bacteria require soft agar in a narrow range of agar concentrations. Media solidified with agar concentrations above 0.3% exclude swimming motility and force the bacteria to move, if possible, over the surface whereas agar concentrations above 1% prohibit swarming of many bacterial species. It is conceivable that the standard 1.5% agar that is used to solidify media in the laboratory might have been specifically chosen for swarming inhibition.

When conducting swarming motility assays, it is necessary to establish a defined set of conditions and adhere to them rigorously[21]. Water content of the media is a crucial factor: too little water will result in poor swarming while too much water may permit swimming motility. To control water content, plates are poured to a standard thickness while the agar is relatively cool (~ 50°C), thereby minimizing water loss from condensation on the plate lid. Finally, swarm plates are dried briefy (~15 minutes) open-faced in a laminar flow hood, to remove surface water and minimize the contribution of swimming motility to surface movement[12,21].

Requirements for swarming motility

Flagella are the most important requirement but swarming also requires an increase in flagellar biosynthesis, cell-cell interactions, and also the presence of a surfactant.

Flagella

Flagella may be observed by phase contrast microscopy using a simple crystal violet-based stain[22], by fluorescence microscopy using fluorescent dyes[23,24], or by electron microscopy[25,26]. The presence of flagellated cells at the front of a spreading colony is consistent with, but not conclusively demonstrative of, the mechanism of swarming motility.

To confirm the mechanism of swarming, mutations causing defects in flagella synthesis or flagella function must abolish colony spreading[27].

Most bacteria that swarm have a peritrichous arrangement of flagella in which multiple flagella are distributed randomly on the cell surface[11,18,25,28,29,30]. Peritrichous flagella bundle together when rotated to effectively increase flagellar stiffness and make force generation more efficient in viscous liquids, a property that may also explain their correlation with swarming[31,32,33,34]. Recently, *E. coli*, which is peritrichously flagellated, has also been shown to swarm between two closely-opposed fixed surfaces[24,35, 36,37]. As a single flagellum requires minimal resource investment and is sufficient for swimming motility, it is tempting to speculate that the synthesis of multiple peritrichous flagella is a specific adapation to generate force in viscous environments and to swarm over and between surfaces.

The correlation between peritrichous flagella and swarming, however, is not absolute and some bacteria with flagella originating from a single cell pole can swarm. *Vibrio parahaemolyticus*, *Rhodosprillum centenum*, and *Aeromonas* make a single polar flagellum that is sufficient to swim in liquids but must induce peritrichous flagella (also called lateral flagella) to swarm over surfaces[28,30,38,39,40]. The polar and lateral flagella are encoded by different genes, powered by separate motors, and are regulated differently[30,39,40,41,42]. *Pseudomonas aeruginosa* is a short, rod shaped bacterium that also makes a polar flagellum. During swarming, *P. aeruginosa* retains its polar flagella but synthesizes an alternative motor specifically required to propel movement on surfaces and through viscous enviroments[43,44]. Thus the expression of alternative motors is at least one other way to facilitate swarming motility besides the use of peritrichous flagella.

When cells transition from swimming to swarming, the number of flagella on the cell surface increases. Organisms with alternative flagellar systems become hyperflagellate in the transition from the single polar to multiple peritrichous flagella. Species with one flagellar system also seem to increase the number of flagella on the cell surface during swarming[6,18,20,25,29,45,46,47]. Even *P. aeruginosa* that swims with a single polar flagellum may produce two polar flagella when moving on a surface[48,49]. Mutations that reduce flagellar gene expression reduce flagellar number and reduce or abolish swarming[17,46,20,50,51,52,53,54,55,56]. Conversely, mutations that enhance flagellar expression increase flagellar number and enhance swarming[47,54,55,57,58,59,60]. The reason that swarming requires multiple flagella on the cell surface is unknown.

Rafting

Whereas bacteria swim as individuals, swarming bacteria move in side-by-side cell groups called rafts[11,17,20,24,26,29,36,49,61,62,63] (Figure 3a). Raft formation is dynamic: cells recruited to a raft move with the group whereas cells lost from a raft quickly become non-motile. The dynamism in cell recruitment and loss suggests that no substance or matrix maintains raft stability save perhaps the flagella themselves. Indeed, scanning electron microscopy of a swarm of *Proteus mirabilis* revealed extensive rafting and perhaps intercellular bundling of flagella[26] (Figure 3b). As with hyperflagellation, the reason that swarming motility requires raft formation is at present unclear.

Surfactant synthesis

Many swarming bacteria synthesize and secrete surfactants (short for “surface active agent”). Surfactants are amphipathic molecules that reduce tension between the substrate and the bacterial cell to permit spreading over surfaces. Surfactants often manifest as a clear, watery layer that precedes the cells at the swarm front[11,29,45,49,64]. Some bacteria fail to

make surfactants and will only swarm on special agar with inherently low surface tension owing, perhaps, to the presence of a surfactant in the agar itself[9,18,35,40,65].

Detecting the presence or absence of secreted surfactant is relatively simple by using a drop-collapse assay[66,67]. When water is spotted onto a hydrophobic substrate (such as polystyrene) the surface tension of the water will maintain the drop as a rounded bead. If a surfactant is present however, hydrophobic parts of the molecule associate with the surface whereas hydrophilic parts of the molecule associate with the water, causing both surfactant and water to spread farther and cause the drop to "collapse". To test for surfactants, culture supernatants need only be spotted on a hydrophobic surface and the degree to which the drop collapses is correlated with surfactant strength and concentration.

B. subtilis and *Serratia liquefaciens* secrete the potent lipopeptide surfactants surfactin and serrawettin, respectively[6,11,68,69,70] (Figure 4). Both lipopeptides are made of a non-ribosomally assembled polypeptide closed into a ring by a fatty acid, and are synthesized by homologous sets of enzymes[6,69,70,71]. Mutations that abolish surfactant production also abolish swarming, and swarming can be rescued by exogenous addition of purified surfactant[11,16,68]. *P. aeruginosa* uses a surfactant that is different from the lipopeptides. Initial characterization of *P. aeruginosa* implicated rhamnolipids as the swarming surfactant[48]. Di-rhamnolipid is composed of two rhamnose sugars attached to the complex fatty acid β-hydroxydecanoyl-β-hydroxydecanoate (HAA)[72,73,74] (Figure 4). Subsequent investigation has shown that the di-rhamnolipid precursors HAA and mono-rhamnolipid also act as surfactants to promote swarm expansion[72,74,96]. The specific properties and potential antagonistic effects of HAA and rhamnolipid molecules during swarming continue to be investigated.

Surfactant production is commonly regulated by quorum sensing[68,75,76,77]. Surfactants are shared secreted resources and are only effective at high concentration. Therefore, quorum sensing may have evolved to regulate surfactant production to ensure that the surfactants are only made when there are sufficient bacteria present to make surfactants beneficial.

Both *E. coli* and *S. enterica* seem to swarm without surfactants. Lipopolysaccharide (LPS), a complex lipid and polysaccharide hybrid in the outer membrane of Gram negative bacteria, was implicated as an important wetting agent because mutations that abolished LPS also abolished swarming[65]. Consistent with a physical role for LPS, surface spreading could be restored to LPS mutants, when introduced to a highly wettable surface or the presence of exogenously provided surfactant[65]. Recently swarming was restored to *E. coli* LPS mutants by secondary mutations in the Rcs envelope stress-response signal transduction pathway that controls flagellar gene expression[63,78]. LPS mutants are also abolished for swarming in *P. mirabilis* owing to reduced flagellar synthesis and swarming can be similarly restored by mutations in the Rcs system[56]. Genetic bypass indicates that LPS is dispensible for swarming, that LPS does not act as a wetting agent, and is instead either directly or indirectly regulatory. The wetting agent that promotes *E. coli* swarming remains unknown.

Swarming-associated phenotypes

The phenotypes of the swarming lag, cell elongation, and colony pattern formation are associated with swarming motility but can be abrogated or bypassed without loss of swarming behavior.

The swarming lag

A lag period of non-motile behavior precedes the initiation of swarming motility when bacteria are transferred from a liquid medium to a solid surface[11,61,79,80] (Figure 5a). The

swarming lag is constant for a particular set of conditions but may be shortened by increasing inoculum density or abolished by using particular mutants[11,54,58,81,82,83]. The lag is poorly understood but its presence indicates that swimming cells must somehow change to become swarming proficient.

There seems to be at least three requirements to exit the swarming lag in *B. subtilis*. The first requirement is for high cell density to induce surfactin production. Surfactin does not determine the minimum lag duration, however, because the lag is not reduced when cells are inoculated on agar that is preconditioned with surfactant[11]. The second requirement for exiting the swarm lag seems to be hyperflagellation because the lag is abolished in cells artificially upregulated for flagellar synthesis[54]. The third requirement is poorly understood and inferred from the fact that the lag is abolished when one harvests actively swarming cells from a plate and reinoculates those cells at high density on fresh swarm media (Fig. 5a). A cell density dependent lag period will reappear, however, when surface harvested swarming cells are diluted and reinoculated in the presence of surfactant (Figure 5b). Thus, the third requirement may represent a critical density of cells necessary to form nucleation centers for the dynamic multicellular rafts reminiscent of the critical protein concentration for the assembly, and the dynamic instability, of tubulin[84,85].

Cell elongation

It is a commonly held belief that swarming cells suppress cell division, and that cell elongation is either a requirement for, or an indicator of, swarming motility. The connection between filamentation and swarming motility originates with *P. mirabilis* which makes short rods when grown in broth and long filaments with multiple nucleoids when grown on surfaces[25,80,86] (Figure 3b). Other bacteria were later found to have subpopulations of long cells enriched at the leading edge of a swarm[18,29,45,87,88]. To date, it is unclear whether elongated cells are required for swarming or whether they simply accumulate at the swarm edge. Despite the importance of elongation in the dogma of swarming motility, no mechanistic or regulatory connection has been elucidated for cell division control during swarming at the molecular level. Furthermore, significant cell elongation is neither a requirement for, nor is it co-regulated with, swarming motility in many bacteria[11,16,39,40,48,49,89,90].

Few studies, beyond the original observations in *Proteus*, have actually confirmed that the elongated cells observed during swarming are, in fact, filamentous. “Filamentous” describes a defect in cell division in which cells continue to grow in the absence of septation. Apparently elongated cells can also arise by a failure of cell separation following successful division resulting in cells linked end-to-end in long chains. Chains and filaments can be difficult to distinguish by phase contrast microscopy but can be differentiated by fluorescence microscopy and membrane staining (Figure 6). Before declaring that a cell is filamentous, one should determine whether or not septa are present.

Colony pattern formation

Swarming bacteria form macroscopic colony patterns on solid media. The patterns may take different appearances but the significance of any particular pattern is unclear. Furthermore, it seems likely that all swarming bacteria can produce a range of patterns depending on the environmental conditions[91,92]. Therefore, pattern formation may be less of a commentary on swarming regulation and more of an indicator of environmental factors.

Featureless swarms are made when cells spread evenly and continuously outward from the point of inoculation as a monolayer. The monolayer is transparent but may be seen when incident light is reflected off the surface or when oblique light is transmitted through the

agar. Cell density in the monolayer is high and roughly uniform throughout the swarm, increasingly slightly at the advancing edge[36]. When the monolayer reaches the boundaries of the plate, the colony grows into a featureless mat[11,20] (Figure 7a).

The most famous irregular swarming pattern is the characteristic bull's-eye formed by *P. mirabilis* that results from cyclic and synchronous waves of motility followed by regular periods of swarming cessation[80,81,93] (Figure 7b). Each cycle produces a macroscopic “zone of consolidation” or “terrace”. In *P. mirabilis*, terraces are thought to arise owing to differentiation into swarming filamentous cells followed by periodic and synchronous de-differentiation into non-swarming short cells[80]. The terraces of *Proteus vulgaris*, however, formed in spite of the fact that the cells remained constitutively elongated[94,95]. *S. marcescens*, and particular mutants of *B. subtilis* also form terraces, but the relationship of terracing to cell shape has not been studied in these cases[52,62].

Dendrites (aka tendrils) are long thin regions of colonization emanating from a central origin (Figure 7c). Dendrite formation in *P. aeruginosa* depends on secretion of of multiple surfactants[72,74,96]. Rhamnolipid derivatives contribute to colony structure differently as the HAA precursor acts as a repellent and fully synthesized di-rhamnolipid acts as an attractant[96]. It is thought that dendrites of *P. aeruginosa* will expand and repel each other as a result of the complicated interplay between the two secreted molecules[72,96]. *B. subtilis* swarms as dendrites under some media conditions and perhaps dendrites arise when the local rates of motility exceed the rate of bulk population growth[12,64]. Dendrites also commonly arise from sliding motility[6,7,8,9,10].

Some bacteria form spiraling vortices as they travel across the surface of the plate[2,62,89,97] (Figure 7d). These vortices are large, localized groups of cells traveling in a common circular path and have also been referred to as “wandering colonies”[2]. In the case of *Paenibacillus vortex*, swarming motility combined with inherently curved cell morphology may produce the vortex pattern[89]. Consistent with an influence of cell shape, *B. subtilis* does not normally make vortices during swarming but will do so when mutations result in long aseptate filamentous cells[98]. Therefore, the vortices may simply be the consequence of constraining swarming to conform to aberrant cell morphology.

Non-swarming cells unable to spread across the surface grow as a confined colony in the center of the plate (Figure 7e). On prolonged incubation, the colony diameter of a non-swarming strain may increase owing to the contribution of sliding motility. The selective pressure for suppressor mutations that restore motility to non-swarming strains is strong. Suppressors segregate from the competition of the colony in asymmetric flares and exploit the uncolonized agar for a massive growth advantage[52,54,82] (Figure 7f). Putative suppressors should be clonally isolated from flares and retested for swarming to determine whether or not they have genetically inherited the ability to swarm. Suppressors may arise rapidly and thus it is advantageous to characterize the swarming defect of a mutant over a limited timeframe with a quantitative swarm assay rather than simply inoculating the center of a plate and incubating overnight[11,52].

Swarming mysteries and controversies

The role of chemotaxis

Chemotaxis is the directed movement of an organism with respect to a chemical gradient. Bacteria mediate chemotaxis by biasing the duration spent in one of two behaviors, either running in a relatively straight line or tumbling erratically to acquire a new direction. Running and tumbling are controlled by the direction in which the flagella rotate. A series of

chemotaxis signal transduction proteins detects stimuli in the environment, transduces the stimulus, and controls the direction of flagellar rotation[99].

Swarming bacteria migrate rapidly away from the point of inoculation and one might assume that swarming behavior is chemotactically oriented because the movement resembles the chemotactic behavior of bacteria swimming through a loose agar substrate[100,101,102]. Furthermore, some swarming bacteria have been shown to be proficient for chemotaxis towards particular chemicals[103,104] and phototactic towards light[38,105]. Finally, some mutants that are defective in chemotaxis also lose the ability to swarm[11,18,102,104,105]. Despite these phenomenological and genetic data, chemotaxis is unlikely to drive bulk swarm expansion because cells in a swarm do not exhibit the running and tumbling behavior that forms the basis of chemotactic orientation and are instead randomly reoriented by external collisions with other bacteria[36]. In addition, swarming is unaffected when chemotaxis is abolished by saturating receptor proteins with non-metabolizable analogs[106] and some mutants severely defective in chemotaxis are not impaired for swarming[11,82,107].

The role of chemotaxis is further complicated by the fact that the chemotaxis signal transduction proteins are often required for swarming in ways that are apparently unrelated to the control of directed movement. One model suggests that the subset of chemotaxis mutants that cause excessive tumbling physically disrupt the ability to form stable multicellular rafts[11,107]. Another model proposes that the chemotaxis system maintains periodic switches in flagellar rotation necessary to somehow extract water from the substrate[90]. A third model invokes the idea that Che proteins have a second function involved in regulating flagellar gene expression and/or flagellar assembly[45,108]. Although chemotaxis proteins are sometimes required, the outward expansion of swarming bacteria seems to be a rapid, non-directed means of distributing a bacterial population over a surface.

The mechanism of surface sensing

Swarming motility requires contact with a solid substrate. Interaction with a surface may induce cells to become swarming proficient during the swarming lag. If surface contact is indeed an inducing stimulus, it stands to reason that the cells must contain a signal transduction system to transduce this information. Elucidating the mechanism of surface sensing, or determining the molecular basis for the bacterial sense of touch, is the “Holy Grail” of swarming motility research.

The sense of touch is poorly understood for all systems but it is particularly problematic for the bacteria. The plasma membrane contains signal transduction systems but is separated from the site of surface contact either by the thick peptidoglycan of the Gram positive bacteria or the de-energized outer membrane of the Gram negative bacteria. Therefore, polymers that transit these layers may provide a conduit for signal transduction and bacterial flagella are potential candidates for a surface sensor. In *V. parahaemolyticus*, the single polar flagellum was implicated as a sensor when inhibition of the polar flagellum by contact with a surface or a range of other means activated lateral flagellar gene expression[28,79,109,110,111]. When flagellar rotation is impeded by contact with a surface, cells may sense changes in ion flux through the flagellar motor[110,111]. Alternatively, cells may sense torque stress on flagellar rotation perhaps through a poorly understood flagellar-associated transmembrane protein called FliL[112,113,114].

The mechanism of force generation

During swimming motility, peritrichous flagella on one cell coalesce into a bundle and rotate to propel the bacterium as a run. A swimming cell tumbles when as few as a single

flagellum changes direction of rotation. Swarming bacteria run but do not tumble and instead occasionally back up when all flagella in the cell reverse direction of rotation and the cell moves backward through the flagellar bundle[37]. Furthermore, swarming occurs in multicellular groups, and it is not known why the same flagella sufficient for propulsion of single cells in liquid are not sufficient for propulsion of single cells on surfaces. Perhaps rafting promotes flagella bundling between cells. If so, how is flagellar rotation coordinated between cells to promote unidirectional movement and raft stability? How is flagellar rotation coordinated in cells to result in direction reversals? How are an increased number of flagella rotated at high cell density without tangling or breaking? New advances in flagellar imaging of individual cells in a swarm will hopefully resolve the mechanism of group propulsion[24,37].

Swarming as a developmental state

Swarming motility is a behavior. Occasionally, the description of swarming motility becomes entangled with the observation of long and hyperflagellated cells that suggest a developmental program. Indeed, the long and short forms of *P. mirabilis* seem to be physiologically different[86,115,116]. Other bacteria experience transcriptional and proteomic changes in contact with a surface but these changes are mostly related to metabolism and stationary phase and flagellar gene expression is unaffected[117,118,119]. Furthermore, cells do not seem to be developmentally “committed” to the swarming state and tend to rapidly lose swarming character when transferred to broth[79]. The swarm lag indicates that swimming cells change prior to becoming swarming proficient but it is not clear that swarm cells constitute a true developmental state.

“Swimming in two-dimensions?”

Researchers who study swarming are often asked: “How do you know that swarming isn't simply swimming motility constrained in two-dimensions?” The possibility that swarming is an artifact of swimming is difficult to dismiss as both behaviors often require the same flagella and there are exceptions to the swarming requirements presented earlier. For example, the apparent increase in flagellar number per cell (hyperflagellation) during swarming has been speculated to be an optical illusion in some bacteria[87,117]. Furthermore, rafting may be a consequence of, rather than a requirement for, swarming because individual *E. coli* cells occasionally move independently of rafts, and rafts may arise passively when an individual's movement is forced to conform to its neighbors[36]. Much of the recent swarming literature comes from studies of *E. coli* and *S. enterica*, powerful model systems for swimming motility, that have among the most conditional swarming phenotypes. It will be important to determine how the swarming of *E. coli* and *S. enterica* relates to the swarming of other bacteria.

Future Directions

For those who are convinced swarming motility is a separate and distinct behavior, many questions remain: What physiological changes takes place during the swarming lag? Is surface contact a direct stimulus and if so, how is it transduced? Is cell division coupled to swarming and if so, what is the mechanistic connection? How is force generated and coordinated in multicellular rafts? How many bacterial species are swarming proficient and how many times has swarming been bred out of laboratory isolates? Finally, what is the ecological relevance of swarming motility? Although the perfect surface of a carefully dried agar plate is never found in the environment, swarming may occur on nutrient rich, soft substrates such as hydrated soils, plant roots, and animal tissues, and swarming cells enjoy a variety of advantages.

Antimicrobial surfactants

In addition to promoting swarming motility, surfactants are potent antimicrobials[120,121,122]. Therefore, swarming motility may be a take-and-hold strategy, in which the same surfactants used to spread across the surface of an object also simultaneously prevent colonization and growth by competing microorganisms.

Bioremediation

Surfactants enhance bioavailability by increasing the solubility of hydrocarbons or by increasing the surface hydrophobicity of hydrocarbon consumers[123,124,125]. Hydrophobic compounds are often surface-associated and therefore surfactants and swarming may aid bacterial nutrition[123].

Pathogenesis

Movement over surfaces may enable bacteria to migrate over, adhere to, and disperse from, sites of infection[26,39,126,127]. Swarming may protect pathogens from macrophages as swarm cells were shown to have enhanced resistance to engulfment[128]. Finally, toxin secretion is often co-regulated with swarming motility[126,129].

Enhanced antibiotic resistance

Bacteria of diverse species seem to become resistant to a broad range of antibiotics when swarming[130,131]. The mechanism of generalized multidrug resistance seems unrelated to known active antibiotic efflux systems and rather is likely passive owing to rapid spreading of cells at high density[118,130,132]. Nonetheless, some bacteria have specialized systems to resist their own secreted surfactants[52,132,133]. The structure of cationic peptides like polymyxin B is surfactant-like, and bacteria may express some antibiotic resistance systems to avoid autotoxicity during swarming[134] (Figure 4).

The study of swarming motility promises novel insights into the bacterial physiology of multicellular behavior. New swarming specific genes await discovery and investigation. New biochemical mechanisms are needed to connect swarming phenotypes to older, better-understood cell physiology. Swarming offers cytological insight into how flagellar number is controlled, biophysical models of how flagella function at a surface, and powerful evolutionary selection pressure. As microbiologists become ever more interested in life at a surface, bacterial swarming motility will surely move the field.

Acknowledgments

I am grateful to Bob Belas, Howard Berg, Eric Déziel, Rasika Harshey, Dave Kysela, Linda McCarter, George O'Toole, Phil Rather, Rivka Rudner, Josh Shrout, and Doug Weibel, for thoughtful discussions about swarming motility and critical reading the manuscript. Work in the Kearns laboratory is supported by the US National Institutes of Health grant GM093030.

Glossary terms

Planktonic	Bacteria growing as dispersed individuals in a liquid environment.
Flagella	The motor for swimming and swarming motility. Flagellar are complex molecular machines assembled from over 40 different proteins. Rotation of a membrane anchored basal body rotates a long, extracellular, corkscrew shaped filament that acts like a propeller to generate force.

Type IV pili	The motor for twitching motility. Proteinaceous pili that extend from one pole of the cell, attach to a surface, and retract. Retraction causes the cell body to move towards the anchor point of the pilus.
Focal Adhesion complex	A putative motor for gliding motility. A putative cell-surface associated complex that anchors a bacterium to a substrate. When coupled to an internal motor, the cell body moves relative to the focal adhesion complexes.
Hyperflagellate	An adjective describing a bacterium that has increased the number of flagella on the cell surface.
Surfactant	A secreted molecule that associates with a surface and acts like a lubricant to reduce surface tension.
Quorum sensing	A strategy by which bacteria regulate gene expression in a manner that is dependent on high population density.

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Box 1: Misnomers

The term swarming motility refers to the verb “to swarm” meaning “to move about in great numbers” because individuals move rapidly in a larger group. The image of a swarm, however, is appropriate for a range of bacterial phenomena and the use of the term “swarm” in the broad sense has caused considerable confusion with respect to the formal definition of swarming motility.

Swarm assay of bacterial chemotaxis

A particularly unfortunate misnomer is found in the common vernacular of the chemotaxis of swimming bacteria. Bacteria inoculated in the center of a nutrient rich plate fortified with less than 0.3% agar will consume nutrients locally, generate a nutrient gradient, and will chemotax up the gradient through the pores in the agar[100]. Although bacteria technically swim through liquid filled pores, the assay is called a “swarm assay”. When reading the swarming literature, it is important to confirm that the agar concentration being used is greater the than the 0.3% needed to exclude swimming and define swarming motility.

Swarmer cells of Caulobacter crescentus

C. crescentus is a bacterium that grows with a remarkable dimorphic life cycle[135]. Each round of cell division is asymmetric and gives rise to a non-motile “stalked cell” that synthesizes a prosthecum with an adhesive holdfast at the tip, and a “swarmer cell” that synthesizes a single flagellum and swims in liquid environments. *C. cresentus* swarmer cells have not been demonstrated to exhibit swarming motility on solid surfaces.

Swarms of Myxococcus xanthus

M. xanthus is a predatory surface-associated bacterium that moves in large multicellular groups and secretes digestive enzymes to destroy and consume other bacteria in the environment[136]. Groups of *M. xanthus* are referred to as “swarms” despite the fact that neither of the independent mechanisms by which they move over surfaces (twitching and gliding) require flagella or constitute swarming motility.

Online Summary

Swarming motility is operationally defined as multicellular, flagella-mediated, surface migration. Swarming also requires an increase in flagellar number, intercellular interactions, and surfactant secretion.

Swarming motility has often been genetically bred-out of laboratory strains and is best observed in natural isolates. In the lab, one must take care to standardize swarming conditions. Although the specific conditions that promote swarming are species dependent, swarming generally occurs on nutrient rich media solidified by agar concentrations greater than 0.3%.

A period of non-motility, or swarm lag, will manifest when cells are transferred from liquid to a solid media. The lag is thought to indicate a physiological change in cells to become swarming proficient.

Some bacteria become elongated during swarming. It is not clear whether cell elongation is required for, or simply co-regulated with swarming, in these species. The mechanistic connection between swarming motility and cell elongation is unknown, and many swarming bacteria do not become elongated.

Swarming often requires the chemotaxis sensory transduction system in ways unrelated to chemotaxis, or directed movement, per se.

The mechanism of surface sensing, or the bacterial “sense of touch”, is unknown but swarming motility provides a strong model system for its study. Models have been proposed to explain response to surface contact including sensing resistance on flagellar rotation when impeded by surface contact, and sensing perturbations in the Gram negative outer membrane.

The ecology of swarming is unknown but swarming is often associated with pathogenesis. Swarming bacteria also enjoy enhanced resistance to antibiotics, enhanced resistance to eukaryotic engulfment, and enhanced nutrition and competitiveness from secreted surfactants.

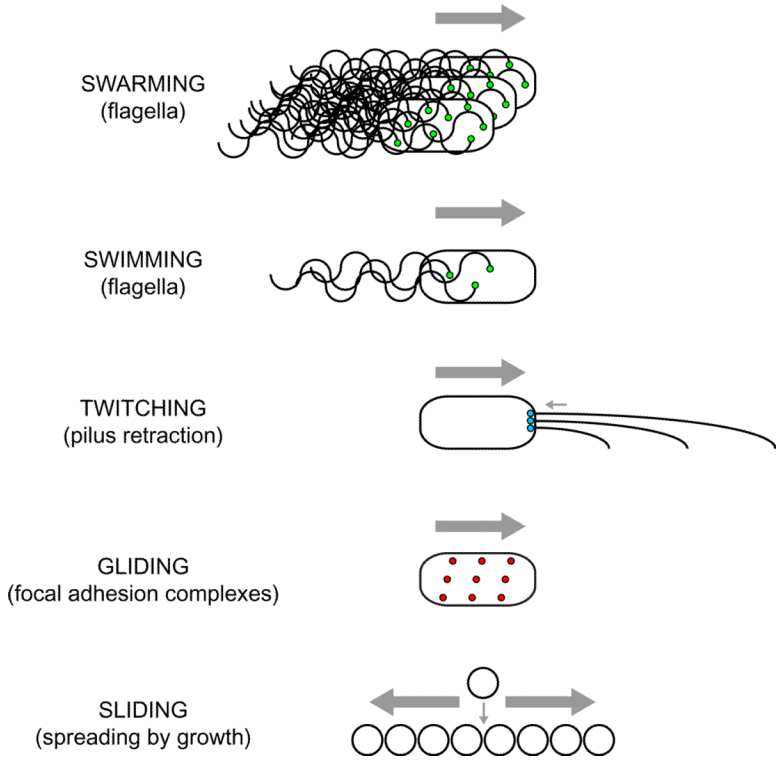

Figure 1. Bacteria move by a range of mechanisms

Swarming is multicellular surface movement powered by rotating helical flagella. Swimming is individual movement in liquid powered by rotating flagella. Twitching is surface movement powered by the extension and retraction of pili. Gliding is active surface movement that does not require flagella or pili and involves focal adhesion complexes. Sliding is passive surface translocation powered by growth and facilitated by a surfactant. The direction of cell movement is indicated by a gray arrow and the motors that power the movement are indicated by colored circles.

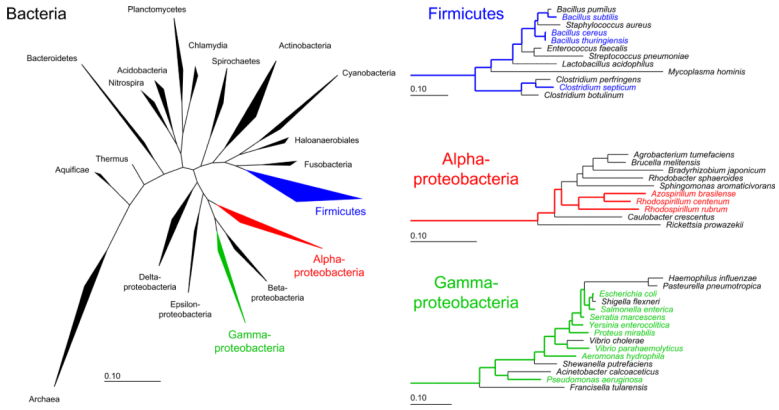

Figure 2. Phylogenetic distribution of swarming motility

A Bacterial phylogeny based on the 16S rRNA gene. Species names in colored text indicate the presence of swarming motility. Species names in black text indicate bacteria for which swarming motility has not yet been demonstrated. Trees generated by Dr. Dave Kysela from 1547 aligned positions using the neighbor joining algorithm on distances determined under the HKY85+I+G substitution model in PAUP* v4.0b10. Scale bar corresponds to a distance of 0.1 substitutions per site.

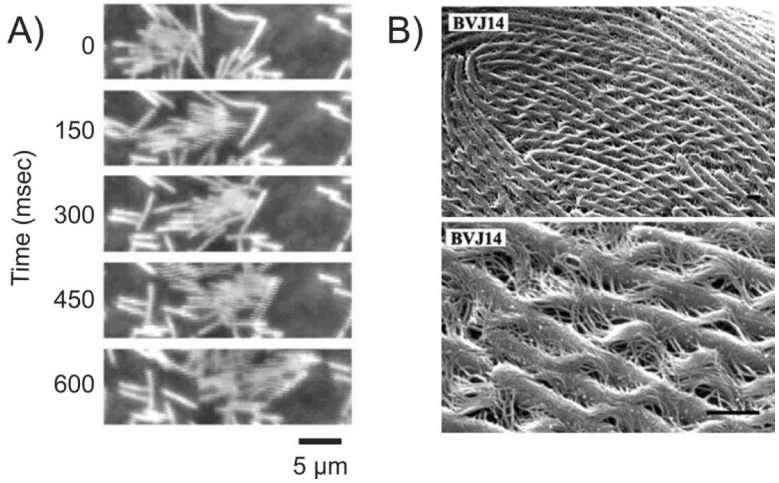

Figure 3. Rafting

A) A timelapse series of images of a raft of *B. subtilis* cells moving in a swarming monolayer. Images cropped from movie S3 published in reference[11]. B) Images of elongated *P. mirabilis* cells swarming as a large raft in a catheter. Panels taken from figure 3 of reference[137]. See also reference[26].

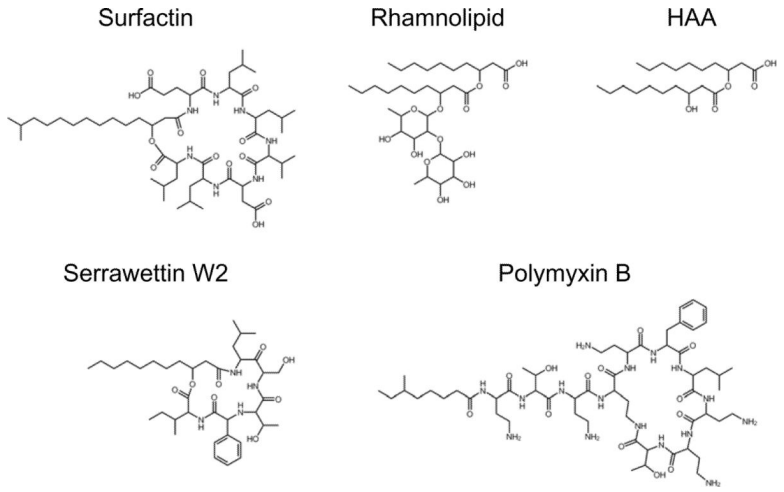

Figure 4. Surfactants

Swarming bacteria use chemically distinct secreted surfactants to spread over solid surfaces. Polymyxin B is an antibiotic that is included here for comparison to the swarming surfactants.

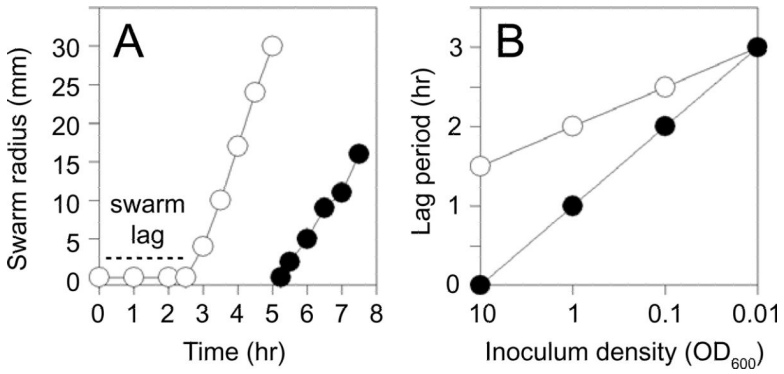

Figure 5. Swarming lag

A) A lag precedes active swarming of *B. subtilis* when bacteria are transferred from broth culture to a solid surface (open circles). The lag is abolished if actively swarming cells are re-inoculated onto a fresh surface (closed circles). Data reproduced from reference[11]. B) The lag period of *B. subtilis* decreases with increasing cell density whether broth grown (open circles) or actively swarming cells (closed circles) are used as inoculum when saturating amounts of purified surfactant are added to the plates prior to inoculation.

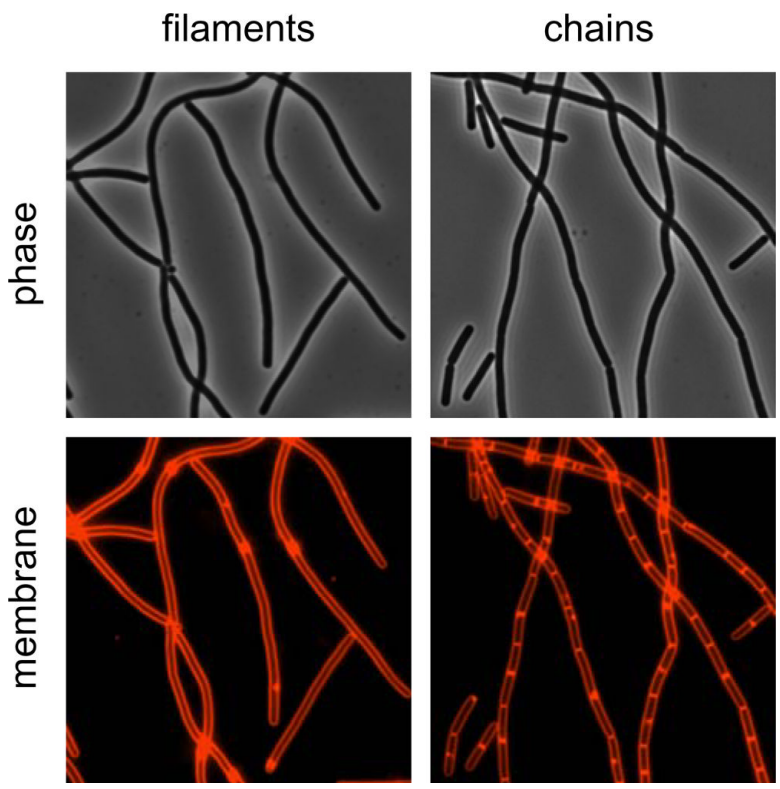

Figure 6. Cell filaments and cell chains

B. subtilis mutant cells are compared using phase contrast and fluorescence microscopy (membrane dye FM4-64, false colored red). Chains of cells (from a *swrA* mutant[54]) have regular septa whereas filaments (from a *minJ* mutant[98]) do not.

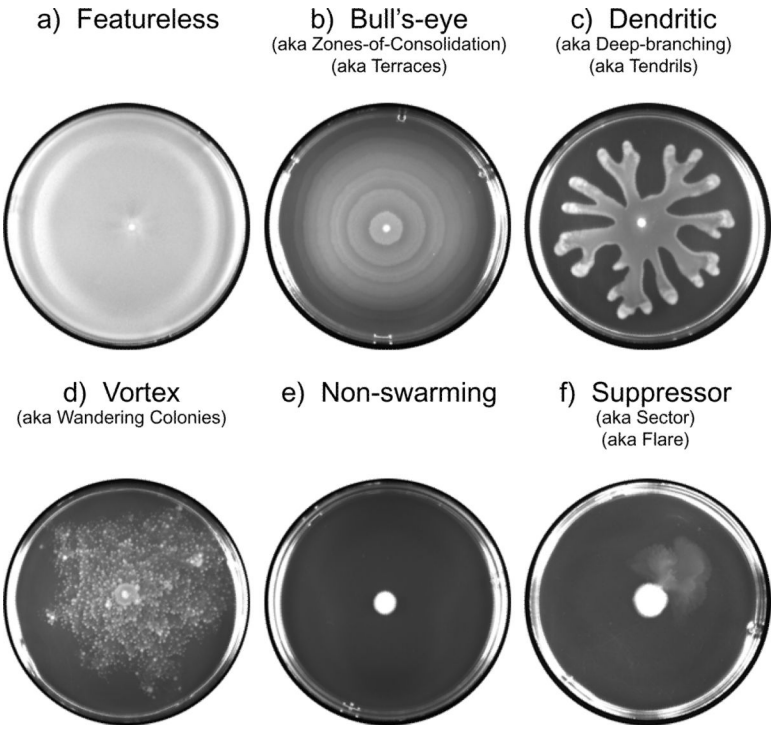

Figure 7. Swarming pattern formation

Featureless: *Bacillus subtilis* 3610, Bull's eye: *Proteus mirabilis* PM7002 (generous gift of Phil Rather, Emory University). Dendritic: *Pseudomonas aeruginosa* PA14 (generous gift of George O'Toole, Dartmouth College). Vortex: *Paenibacillus vortex* V (generous gift of Rivka Rudner, Hunter College). A non-swarming mutant and subsequent suppressor in *Bacillus subtilis* 3610. Uncolonized agar appears black and bacterial biomass is white.